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NUTRITION REVIEWS

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

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FUTURE STUDIES IN NUTRITION

In attempting to outline some of the problems in nutrition which need our attention during the next ten to twenty-five years, it seems appropriate to quote the statement made in the Report of the Scientific Director of the Nutrition Foundation: "In brief we want to find what quantity of each of the forty or more essential nutrients is required for good nutrition, how each nutrient functions inside the body, how each can be used to protect human and animal health, and how each can be measured accurately, either as it functions in the body or as it comes into being on a farm and later takes its course through a modern factory." That covers about everything we need to do.

The term "Food and Nutrition" is widely used these days, and indicates, I think, that we must recognize two important sections of nutrition: first, the production and handling of food, and second, the utilization of this food in natural or modified form for optimum health. It is interesting that a group of industries concerned with the production of food designated their Foundation the "Nutrition Foundation." It is clear that they are mainly interested in producing food for the maintenance of optimum health.

It is evident to all of us that we have learned much about nutrition from studies on food. The early protein requirements were based upon the amount of protein in diets consumed by average human subjects. Chittenden questioned the high intake on the basis of self indulgence but today we are more willing to accept the higher intakes. Sherman used similar methods for estimating calcium and iron requirements. Even our more recent dietary allowances are questioned if the suggested amount of a given nutrient cannot be met by an ordinary diet. I am inclined to believe that this old-fashioned method of determining requirements by measuring food consumption of certain groups may be one of our best procedures.

We must, therefore, know what chemical compounds are present in all our foods. Biochemists might organize on a factory scale and attempt to isolate and identify every compound in biologic material just as organic chemists are interested in synthesizing a variety of compounds in hopes that some use will be found for them. It would be a difficult job to do this in the case of liver. Such a scheme is not impossible but we will undoubtedly continue to see greatest interest in those compounds having biologic significance. One of the penicillin experts has told me that no chemist would have thought of a compound like penicillin if the biologic activity had not attracted attention to it.

We certainly have some carbohydrate-like compounds and many fat-like substances left to isolate. We may be familiar with most of the amino acids but we have many proteins left to isolate, especially the enzymes and the viruses. We need to look for peptide-like compounds, such as strepogenin which Woolley has found in casein and trypsinogen but not in egg white. We still do not understand the peptide-like compounds related to the pernicious anemia factor. We need to know more about the remaining mineral elements in food both from the point of view of their beneficial effect and their possible toxic effects. New analytical procedures will probably be needed. It is easy to imagine the earlier application of fluorine therapy in dental caries if methods for the detection of fluo-

rine in water had been available at an earlier date.

We must be approaching the end of our list of vitamins but I doubt that anyone is willing to agree that none remains to be identified. But even so there are still some of the so-called vitamers left to untangle. How many "folic acids" do we have? Some of you may ask how we can find more vitamins when rats will now grow on a diet containing only the known vitamins. It is necessary to use different types of animals. Cows and pigs were used in the early studies on vitamins A and D and calcium. The rat was somewhat more critical in its needs but even so six B-vitamins have now been found to be sufficient. The rat's dietary requirement for biotin and "folic acid" can be brought out only by the use of sulfa drugs. The dog does very well on six B-vitamins even in the presence of sulfa drugs, which indicates that the intestinal flora synthesizing the remaining factors must be well established in the tract of dogs. The chick requires both biotin and "folic acid" for growth. Recent work has shown that 25 micrograms of synthetic "folic acid" or L. casei factor per 100 g. of ration will produce good growth, good feathering, and a good blood picture, but that does not mean that synthesis of additional factors may not occur when this level of "folic acid" is supplied. The monkey also requires biotin and "folic acid," and under certain conditions an additional factor present in whole liver is necessary for the prevention of anemia. This factor is very labile and over half of the activity in fresh liver may be lost during ordinary drying procedures. Guinea pigs will also give us much information about new factors if we can find ways of producing pigs that are not infected.

To date most of our work has been done with growing animals, but I am sure there are more factors in store for those who carry their animals through reproduction. Rats, dogs, and pigs have shown reproduction failures on diets considered adequate for normal growth. Perhaps we can use animals very early in life. Calves show some interesting responses when handled under different conditions. Many of these effects are undoubtedly related to synthesis of vitamins by the intestinal bacteria. We must interest more bacteriologists in these studies and we should produce sterile animals for nutrition studies as soon as possible.

So far I have considered only those compounds that may have some nutritional significance; it is also important to know more about the compounds that give foods their taste appeal, their flavor, and aroma. Why do some people like liver and other people hate it? Why do some like Limburger cheese while others are nauseated by it? Sooner or later chemists and physiologists must get together on this subject. I am also afraid that most biochemists interested in isolating compounds from biologic material have tried to use raw or unprocessed foods. We need to know what compounds may be formed during processing. What happens during cooking? Are some of the fat decomposition products deleterious? During the war we learned that a number of new compounds were formed in dehydrated foods. The significance of the Maillard reaction was recognized but little is known about the real chemistry of it. In our own laboratory we find that wheat gluten produces running fits in dogs and yet whole wheat fed in an equivalent amount shows no such effect. A new compound must be formed at some stage in the preparation of wheat gluten. Is sufficient sugar produced from the starch to allow its action with certain amino groups to form a toxic compound?

It is not only important to know what is present in our foods, but we must know how much of each of these compounds is present. We have recently been impressed by the fact that we know little about the quantity of different carbohydrates in foods. While we have calculated the energy value of the carbohydrates in foods by multiply-

ing total carbohydrate by 4, a figure suggested by Atwater, the British are insistent upon using available carbohydrates and a different factor for sugar and starch. I do not want to take sides but we have a lot of analytical work to do if we select the British method; it may be important to know the amount of different carbohydrates for other reasons than that of calculating the energy value. In the case of fat, we have not gone much beyond total fat. We have made some attempt to determine sterols and phospholipids but much more analytical work is necessary. During the past few years we have seen a great increase in interest in methods for the estimation of amino acids by chemical, microbiologic, and isotope dilution methods. Many problems still remain, such as methods of hydrolyzing the proteins, the prevention of racemization, and finally methods of expressing the amino acid content of a protein. We feel rather confident about our methods for the estimation of mineral elements but I wonder if we would be so enthusiastic if extensive collaborative studies like those made on vitamins would be carried out on some of our mineral elements. The greatest progress has been made in the case of vitamins and I think we can be proud of the results. However, we still have much to learn about the determination of vitamin A even in such things as liver oils and we still have no simple method for vitamin D. Methods that are simple and more rapid for all the vitamins are bound to be developed. We also have the problem of different forms of the vitamins such as pyridoxine, pyridoxal, and pyridoxamine. This brings us to the relative accuracy of biologic and chemical or microbiologic methods. I feel that we must develop methods aside from the animal assay in all cases. There is no comparison in absolute accuracy and if we have different forms of the same vitamin or amino acid we must know the biologic value of each form and then calculate total activity from the *in vitro* assays. There remains the question of digestibility and availability but difference between total content and available content must be understood. If we know the compounds present we can explain availability. Availability of amino acids in soybeans is dependent upon antienzymes. Most of the studies on soybean proteins have been related to methionine because it was the most limiting amino acid, but antienzymes may effect the liberation of many of the amino acids. Heat treatment may destroy the antienzymes but may also destroy certain amino acids if the heat is not controlled. Recently an antiamylase in navy beans has been reported.

It is unnecessary to emphasize the further need for studies on the preservation and enhancement of the important compounds in foods during food production and processing.

There is need to continue all the routine work necessary for the construction of a truly complete table of food composition. The information on the qualitative and quantitative composition of foods is only a starting point and this information must be integrated and used with a thorough knowledge of the physiology of the living body in order to obtain optimum nutrition. Little can be done without values for the composition of food, but little is accomplished if we stop here. It is also important to point out that nutritional studies are greatly facilitated by having many compounds such as vitamins and amino acids available in pure form. I am afraid few laboratory workers really appreciate the importance of the quantities of pure vitamins and vitamin concentrates which have been supplied gratis by industry for experimental purposes. Our problem therefore becomes one of using all nutrients in our food and in concentrates in proper relationship in order to obtain optimum health.

Optimum health is an important phrase. It means adequate growth during infancy, normal efficiency during active life, normal reproduction, normal longevity, and a complete life with the least discomfort and

disease and the greatest amount of well-being. It is not sufficient that we give generous amounts of each new nutrient. A specific nutrient may be more beneficial at a low level than at a generous level. In order to study these effects we must know what happens to food in our bodies. This means not only the changes occurring in the digestive tract, which is a field that needs tremendous study, but also the reactions that take place in the cells themselves.

One of our biggest fields for future study relates to enzymes and enzyme mechanisms. Recently I attended a conference on intracellular enzymes in normal and malignant tissues at Hershey, Pennsylvania, and I think all of us were astounded to find how little we knew about this subject when we attempted to outline the information which was available at the present time. We must give further consideration to the relation of hormones to these enzyme systems especially in light of Cori's recent demonstration of the relation of insulin and anterior pituitary to hexokinase. When we know how specific nutrients enter into some of these enzyme mechanisms it will help us to establish more definitely some of the nutritional requirements. Thus it is generally established that 100 micrograms of thiamine per 100 g. of ration are sufficient for all types of animals. We have considerable confidence in this figure because we know it takes a definite amount of thiamine for the production of cocarboxylase, which in turn is necessary for the metabolism of carbohydrates and carbohydrate residues. However, this figure does not take into account the possible destruction of thiamine in the intestinal tract or the possible production of thiamine by bacterial synthesis. It does not take into account the possibility that thiamine may help compensate for lack of other factors. We have found in the case of rats that if the ration is low in "folic acid" a higher level of thiamine will help compensate for the lack of "folic acid."

Similarly we may have production of appreciable amounts of certain vitamins in the intestinal tract and these relationships need thorough study. A high lactose diet will increase the synthesis of riboflavin but it may decrease the production of other factors. Fat may decrease the requirement for thiamine but actually reduces the production of riboflavin in the tract. The kind of carbohydrate may have a definite effect on the production of several of the vitamins in the tract. We have recently found, in attempting to produce more clearcut B_6 deficiencies in rats, that when the diet contains ample amounts of biotin, and the carbohydrate is dextrin, the animals grow well with no added pyridoxine.

Many of these studies have been made with purified or so-called synthetic diets. We used to think that all our difficulties would be solved if we could use only diets of known composition in our experimental work. During the past two years we have come to realize that natural diets may give effects quite different from synthetic diets and, after all, it is the natural diet which we must use for human nutrition. I should like to outline briefly some of the work which we have done with seminatural diets, which clearly demonstrates the interrelationship between various nutrients.

If we feed rats a purified diet containing 15 per cent casein and all the known vitamins except nicotinic acid the animals grow rather well. However, if 40 per cent of this diet is replaced by corn grits the rate of growth is greatly reduced. The effect is completely counteracted by the addition of 1 to 1.5 mg. of nicotinic acid per 100 g. of ration. This retardation in growth was not obtained when we used other grains such as barley, oats, and rice, even though some of these grains contained less niacin than corn. After considerable study it became evident that this effect might be related to the low tryptophane content of corn proteins and when we added 50 mg. of tryptophane per 100 g. of ration the growth was normal. In these studies the kind of carbohydrate also influ-

ences the results. Glucose, dextrin, and lactose were more beneficial in their effect than other carbohydrates. Small amounts of milk were helpful in counteracting the growth depression. Low levels of the other vitamins accentuated the deleterious effect of corn. It was also most interesting that diets containing 9 per cent of casein produced fairly good growth in rats but when 6 per cent of gelatin was added in addition to the casein, very poor growth resulted. This retardation can again be counteracted to some extent with either nicotinic acid or tryptophane. All proteins or protein preparations low in tryptophane seem to produce this ill effect when added to diets containing fairly low amounts of protein. We can get the same effect with zein and with an acid hydrolysate of blood fibrin in which the tryptophane is destroyed.

These findings, we feel, are of fundamental significance since they indicate that a diet high in protein, but of poor quality, may be much more deleterious than a diet low in protein, but having a fairly well balanced amino acid composition. Thus we have a great deal to learn about amino acid requirements on various diets, even after we know the complete amino acid composition of all protein foods. It is evident from these preliminary results that any attempt to establish a specific figure for the human requirement of each nutrient is futile. Greater progress will be made if we accept figures in the general range and give more attention to newer factors or interrelationships between the known factors.

One phase of nutrition which needs a great deal of study is that of dental caries. Working under a grant from the Nutrition Foundation we have been able to standardize the cotton rat so that dental caries can be studied with considerable accuracy. Under these conditions we find that diets high in fermentable sugars are much more conducive to dental caries than natural diets or purified diets containing dextrin as the carbohydrate. Furthermore, we have shown that a higher level of protein is protective and, more specifically, a higher intake of fat has a beneficial effect in preventing caries. The effect of protein and fat is additive and is not due merely to the decrease in the amount of fermentable sugar in the diet. We have also found that milk is a protective food and even when we add 10 per cent of sugar to a whole milk diet no caries is produced. You may suggest that milk has some specific effect which we cannot relate to chemical composition. However, I definitely feel that if we learn more about these relationships we can discover the specific chemical substances in milk that are active.

Another phase of nutrition which needs a great deal of work is the relation of nutrition to resistance to infection. It is, of course, well known that any condition leading to a depletion of the protein reserves may depress resistance through reduction in the quantity and quality of the phagocytic tissues and through its adverse effect on the antibody mechanism. However, the relation of virus infection to adequate nutrition is not nearly as evident. We have recently found in the case of the poliomyelitis virus that mice deficient in thiamine, pantothenic acid, sodium, and potassium are much more resistant than litter mate animals receiving adequate quantities of each of these nutrients. This does not mean that anyone wishes to advocate nutritional deficiencies for the purpose of preventing virus infection. However, it does clearly indicate that there is a relationship between nutrition and infection and that these deficiencies may actually help to conserve unknown factors which may be present in critical amounts. In fact, we have recently had some success in preventing infection in monkeys fed a so-called superdiet.

I have said nothing about studies on methods for diagnosing subclinical deficiencies in human beings—not because I do not feel these studies are important, but because I know very little about them, and also,

because I feel work in that line will progress more rapidly if we know more about the nutritional requirements of different types of animals. I should like to emphasize that while human studies are important, we should be careful not to make these studies too extensive before we have the fundamental facts which can be obtained much more readily with experimental animals. Thus there is plenty of indication that nutrition is still a young science and that we have a long hard row ahead of us if we are to have a true, scientific knowledge about nutrition. We have seen sufficient application of our nutritional findings during the past few years to give us considerable satisfaction and also hope that still greater application may be made if we are sure of our scientific information.

I should also like to make a plea regarding the effective use of synthetic food materials such as vitamins and amino acids. This, I believe, is a problem which economists will have to help us solve, but surely the nutritionists will have to take the lead. If synthetic products replace some of our more expensive food products we may produce an economic effect upon agriculture which in the long run will be detrimental to optimum health. However, if these synthetic products can be used to supplement rather than to replace natural foods, we will have a better opportunity for maintaining an optimum diet.

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PRESENT KNOWLEDGE OF PROTEIN IN HUMAN NUTRITION

It would be regarded as a platitude to state that proteins play a fundamental role in nutrition. On the other hand, the elucidation of the extent to which proteins are important must be admitted as being new and in many ways the result of recent investigation. Most of this newer knowledge, although derived from studies in animals, is probably applicable to man. It is well known that proteins are essential constituents of both the protoplasm and the nucleus of cells. It is recognized that they are the main organic constituents of muscle and of the glandular tissues of the body. Their role in regard to the osmotic relations between intracellular and extracellular fluid is appreciated. With increasing understanding of the intricacies of metabolism and nutrition, knowledge has been gained concerning the manner in which proteins play a role of prime importance. Functioning as enzymes, hormones, or antibodies, proteins represent in large measure the essential purpose of various body tissues.

An important advance in our understanding is furnished by the experiments of Madden, Whipple, and their associates (*Nutrition Reviews* **2,** *322 (1944)*). These workers have shown that plasma protein is not static but reflects tissue stores. Furthermore, there is a ready interchange between plasma proteins and hemoglobin, in both directions. To this interchange the expressive term, "dynamic equilibrium" has been applied. From the body protein pool are evolved the needed proteins, whether hemoglobin, plasma protein, or tissue proteins. Studies made with the aid of heavy nitrogen indicate that there is a highly mobile fraction of tissue proteins exchanging constantly with the plasma proteins and approximately equal with the latter in size (*Ibid.* **3,** *137 (1945)*). These experiments, incidentally, also indicated that the rate of mobilization of tissue protein into plasma protein is greatly reduced in shock.

In protein starvation, it appears, the body draws upon the reserve stores of protein and

upon the plasma proteins for its metabolic needs. The protein depleted dog cannot use effectively some of the waste products coming from protein wear and tear (*Nutrition Reviews* **1,** *200* (*1943*)). All dietary proteins have not been proved equally effective as precursors of plasma protein. Fresh and dried beef serum is acknowledged as representing protein of highest potency for plasma protein regeneration and, according to Weech, lactalbumin is of equal value. Egg white, beef muscle, liver, casein, and gelatin follow, in the order named. For hemoglobin production, dog plasma, dog hemoglobin, casein, beef serum, and an amino acid mixture were effective in the order given (*Ibid.* **1,** *284* (*1943*)). Use of these substances for hemoglobin production to a greater extent than for plasma protein formation is explained in part by the fact that in the dog the quantity of hemoglobin is approximately three times the amount of plasma protein. Whether these observations in the dog are wholly applicable to man, remains to be seen. In the dog the ten amino acids essential for growth in the rat, plus glycine, will maintain nitrogen balance and produce as much new plasma protein as will good diet proteins, according to Whipple and co-workers (*Ibid.* **2,** *322* (*1944*)).

The final word concerning the amino acids "essential" for man has not yet been given. Criteria in addition to growth and nitrogen balance may be important. For example, deficiency of a particular amino acid may lead to a specific type of injury; an instance of this is found in the study of arginine deficiency by Holt and his co-workers who noted a change in spermatogenesis. According to Rose, Haines, Johnson, and Warner (*J. Biol. Chem.* **148,** *457* (*1943*)) at least eight of the ten amino acids essential for the rat are necessary for the maintenance of nitrogen equilibrium in young human adults for limited periods. Very little has been learned so far regarding the quantitative requirements for each of the essential amino acids in man.

Stimulated by the problems arising through warfare and pointed up by the striking benefit in the treatment of infections resulting from the use of sulfonamides and penicillin, problems of tissue repair have received attention. Recognition is being given to the fact that tissue injury whether by physical trauma, burns, infection, or major surgical procedures, is associated with nitrogen losses which may be very large. Following trauma, even in the absence of infection, a pronounced negative nitrogen balance develops. In cases of fractures, an average loss of body nitrogen as high as 220 g. has been observed (*Nutrition Reviews* **3,** *86* (*1945*)). This amount represents 1400 g. of protein or 15 pounds of muscle protoplasm. This protein catabolic process cannot be interrupted by increasing the intake of highest quality protein and calories, unlike the reaction in starvation. What physiologic purpose is served by this catabolism, is not clear. In burns, nitrogen is lost by excretion, in addition to the nitrogen in the exudate which forms. In the event of a burn corresponding to half the body of a man of average size, the nitrogen loss may be so great as to correspond to 2000 ml. of plasma (*Ibid.* **3,** *2* (*1945*)). In other types of exudates associated with inflammatory processes, as much as 50 g. of protein may be lost per day. Protein deficiency may lead to edema, delayed wound healing, impaired gastrointestinal function, the development of peptic ulcers, and liver damage.

The role of proteins in the prevention and amelioration of infections is also receiving attention. Experimental studies by Cannon and associates indicate that both the acquisition and the retention of specific resistance may be determined largely by an adequate intake and utilization of dietary protein. It also seems that impairment of the antibody mechanism is one of the sequelae of protein undernutrition (*Nutrition Reviews* **3,** *78* (*1945*)). Antibodies are apparently specifically modified globulins. However, it is necessary to distinguish resistance to

infection, a rather complex reaction, from antibody response—a reaction which attempts to limit the extent of infection. Experimental studies which await confirmation suggest that specific deficiency of certain vitamins and of protein may lead to decreased phagocytic activity of leucocytes (*Ibid.* **2,** *232* (*1944*)).

Elucidation of the role of proteins, and especially of the sulfur-containing amino acids for the functioning of the liver and their importance in maintaining its normal structure, has led to the study from the nutritional standpoint of hepatic disorders in man. These experiments have, in the first place, brought about a change in the concept long held that the administration of large amounts of glucose is the most important measure in the management of patients with hepatitis. It has been reported also that the occurrence of liver damage during arsenical treatment may possibly be minimized or even prevented by the administration of methionine (*Nutrition Reviews* **2,** *340* (*1944*)) and that the course of toxic and infective hepatitis is influenced beneficially by such therapy. Restudy of Laennec's cirrhosis of the liver has shown that besides alcoholism, dietary factors are of importance in its pathogenesis, and that the course of such cases can be greatly benefited by feeding a diet rich in protein and the vitamin B-complex (*Ibid.* **1,** *88* (*1943*)).

It is much too early to determine whether animal studies which indicate that dietary factors, including the protein or amino acid composition of the diet, play a role in carcinogenesis (*Ibid.* **2,** *298* (*1944*)), and also have any relationship to cancer as it appears in man. Diet may no doubt influence the metabolism of malignant as well as of normal cells. Toxic waste products may also play a role. The extraordinarily high incidence of liver cancer in the African Bantu and the extremely poor diet consumed by these natives, have suggested a causal relationship, especially in view of the fact that some degree of cirrhosis of the liver has been found in almost every Bantu with hepatic cancer (*Ibid.* **3,** *19* (*1945*)).

In the light of the demonstration of the role of protein in nutrition, it is only natural that much attention has been given to ways and means for protein administration, especially its parenteral use. It has been possible to maintain normal nitrogen balance and even to correct protein deficiencies by intravenous administration of amino acids or casein hydrolysate. Certain limitations of this procedure are being demonstrated. There seems to be, at least in dogs, an optimal rate of administration from the standpoint of efficient utilization (*Nutrition Reviews* **2,** *74* (*1944*)). For purposes of rat growth it has been found that human plasma is deficient in isoleucine while human albumin is lacking in tryptophane as well (*Ibid.* **3,** *171* (*1945*)). This, together with the fact that protein for parenteral use, unlike that supplied as food, is not accompanied by a rich supply of the vitamin B-complex, including the factors as yet not produced synthetically, limits the usefulness of parenteral protein or amino acid therapy. Nevertheless, as an adjunct in nutrition or as a temporary measure for its effective colloid osmotic activity, such parenteral therapy now has a solid position in therapeutics.

THE UNITED NATIONS FOOD AND AGRICULTURE ORGANIZATION

The first conference of FAO—the Food and Agriculture Organization of the United Nations—which opened at Quebec City, Canada, on October 16, 1945, has a background worth recalling. This organization is the first of the new permanent United Nations organizations to be launched after the war and it is the direct outgrowth of the

United Nations Conference on Food and Agriculture which was held in May, 1943, at Hot Springs, Virginia. An excellent summary of the accomplishments of this earlier conference was provided by Boudreau in this journal (*Nutrition Reviews* **1,** *321 (1943)*).

The Hot Springs Conference decided to set up an Interim Commission consisting of representatives of all of the governments participating in that conference, to implement the recommendations adopted at Hot Springs. One of these recommendations was "that the governments and authorities here represented establish a permanent organization in the field of food and agriculture" (*First Report to the Governments of The United Nations by The Interim Commission on Food and Agriculture, Washington, D. C., August 1, 1944*). The Interim Commission, under the chairmanship of L. B. Pearson of Canada, formulated a constitution for the proposed permanent organization and, after revision, the final draft of this constitution was adopted by more than twenty governments, the number necessary to bring FAO into existence. Hence, invitations were extended to the forty-four United Nations governments to attend the Quebec City Conference, the initial meeting of the permanent organization. With the establishment of the FAO, the Interim Commission was dissolved.

The Constitution of FAO is included in the first report to the governments referred to above. The closeness with which the permanent organization follows the pattern set down by the Hot Springs Conference in 1943 is evident by an examination of this document. Thus, the Preamble of the Constitution of FAO states the reason for its existence as follows:

"The Nations accepting this Constitution, being determined to promote the common welfare by furthering separate and collective action on their part for the purposes of

raising levels of nutrition and standards of living of the peoples under their respective jurisdictions,

securing improvements in the efficiency of the production and distribution of all food and agricultural products,

bettering the condition of rural populations,

and thus contributing toward an expanding world economy,

hereby establish the Food and Agriculture Organization of the United Nations, hereinafter referred to as the 'Organization,' through which the Members will report to one another on the measures taken and the progress achieved in the fields of action set forth above."

The functions of the Organization are defined as follows:

"1. The Organization shall collect, analyze, interpret, and disseminate information relating to nutrition, food and agriculture.

2. The Organization shall promote and, where appropriate, shall recommend national and international action with respect to

(a) scientific, technological, social, and economic research relating to nutrition, food and agriculture;

(b) the improvement of education and administration relating to nutrition, food and agriculture, and the spread of public knowledge of nutritional and agricultural science and practice;

(c) the conservation of natural resources and the adoption of improved methods of agricultural production;

(d) the improvement of the processing, marketing, and distribution of food and agricultural products;

(e) the adoption of policies for the provision of adequate agricultural credit, national and international;

(f) the adoption of international policies with respect to agricultural commodity arrangements.

3. It shall also be the function of the Organization

(a) to furnish such technical assistance as governments may request;

(b) to organize, in cooperation with the governments concerned, such missions as may be needed to assist them to fulfill the obligations arising from their acceptance of the recommendations of the United Nations Conference on Food and Agriculture; and

(c) generally to take all necessary and appropriate action to implement the purposes of the Organization as set forth in the Preamble."

Membership in the Organization consists of those governments whose representatives served on the Interim Commission and who adopted the Constitution. Additional members may be admitted. Forty governments are now members of FAO.

The United Nations Food and Agriculture Organization consists of a conference, an executive committee, and a secretariat. The conference, which will meet once a year, consists of representatives of the member nations, each with one vote. In addition to the single representative, member nations may send an alternate, associates, and advisers. At the Quebec Conference some national delegations consisted of as many as 30 persons.

The Executive Committee consists of 15 persons who act on behalf of the whole conference and not as representatives of their governments. The conference delegates many of its powers to the Executive Committee, which will meet more frequently than the conference and be more closely associated with the work of the organization. Mr. Howard R. Tolley, Chief of the Bureau of Agricultural Economics in the United States Department of Agriculture, is the American member of the Executive Committee.

The unanimous choice of the conference to head the secretariat was Sir John Boyd Orr of Aberdeen who thus became the first Director General of FAO. This choice clearly indicated the conference's decision to make FAO something more than an agency for research and the dissemination of information. Until the seat of UNO is determined the headquarters of FAO will be in Washington, D. C.

The annual budget of FAO is submitted by the Director-general and, after approval by the conference, is allocated among member nations as determined by the conference. The provisional budget for the first fiscal year is 2,500,000 U. S. dollars to be contributed by member nations in proportions ranging from 0.05 per cent for the smallest nations to 25.00 per cent for the United States of America.

The problems facing this organization are great; the contributions to be made by it are immeasurable. For it is even more apparent today than in 1920, that "the experiences of the great war have forced us to realize as never before that the maintenance of the food supply is the basal problem of civilization. Before commerce or manufacturing or mining can be carried on—before science or art or religion can flourish—man must be fed. A starving world cannot be made safe for democracy .... Food must be abundantly produced and rationally consumed as well as honestly and economically distributed" (Armsby, *Yale Review* **9,** *330 (1920)*). It is clear that this fundamental problem is not limited by international boundaries.

FAO is not to be confused with UNRRA, the Director-general of which is Governor Lehman. This temporary relief organization was designed to provide a reservoir of emergency supplies and services which would enable the inhabitants of devastated areas to start again on the road towards decent conditions of living. The chief concerns of UNRRA, therefore, are with meeting emergency needs for clothing, medical supplies, farm equipment, livestock, fertilizers, transportation equipment, and food in order that the populace may survive and inaugurate its own food and material production. FAO

is the first of the permanent United Nations organizations, and it will be one of several agencies whose main task will be to promote international cooperation in meeting fundamental human needs and thus building peace on a solid foundation.

SOME CLINICAL INVESTIGATIONS WITH ISOLATED AND SYNTHETIC L. CASEI FACTORS AND RELATED COMPOUNDS

The recently announced synthesis of an L. casei factor by a large group of investigators from the Lederle Laboratories, Inc., and the Calco Division of the American Cyanamid Co. (Angier *et al.*, *Science* **102,** *227* (*1945*)) has provided a marked impetus to the investigations of the clinical importance of this liver factor. The compound which has been prepared is identical with the L. casei factor which was previously isolated in pure form from liver by Stokstad (*J. Biol. Chem.* **149,** *573* (*1943*); *Nutrition Reviews* **4,** *15* (*1946*)). It is, therefore, equally active for growth of L. casei and S. lactis and is a hematopoietic substance for chicks. The structure of the compound has not been announced, but it has ultraviolet and infrared absorption spectra identical with those of the natural compound. The crystals of the two compounds and the biologic activities are identical.

Three reports of the clinical effectiveness of this newly synthesized factor have been made. In order to understand the logic involved in these trials it is well to review briefly some of the experimental background upon which they rest. Wills and Bilimoria (*Indian J. Med. Res.* **20,** *391* (*1932*)) and Wills and Stewart (*Brit. J. Exp. Path.* **16,** *444* (*1935*)) described the dietary production in monkeys of a macrocytic anemia accompanied by leukopenia, diarrhea, and a megaloblastic bone marrow. Independently, Day, Langston, and Shukers (*J. Nutrition* **9,** *637* (*1935*)) reported a similar syndrome which developed in monkeys on a diet lacking some component of the vitamin B-complex. This syndrome consisted of leukopenia, anemia, diarrhea, oral lesions, and edema and resulted in death. Day, Langston, and Darby (*Proc. Soc. Exp. Biol. Med.* **38,** *860* (*1938*)) further differentiated this factor and suggested the name "vitamin M" for this hitherto undifferentiated factor which prevented nutritional cytopenia in the monkey. These findings were widely confirmed (see Day, *Vitamins and Hormones* **II,** *91* (*1944*)). Liver, liver extract, and yeast were found to be rich sources of vitamin M. Wilson, Doan, Saslaw, and Schwab (*Proc. Soc. Exp. Biol. Med.* **50,** *341* (*1942*)) and Saslaw, Wilson, Doan, and Schwab (*Science* **97,** *514* (*1943*)) emphasized the occurrence of a "folic acid" in vitamin M containing concentrates. These findings were confirmed by Waisman, Rasmussen, Elvehjem, and Clark (*J. Nutrition* **26,** *205*, *361* (*1943*)). Day and collaborators found a parallel distribution in foodstuffs of vitamin M and "potential folic acid" (*Nutrition Reviews* **4,** *15* (*1946*)). Finally, Day and collaborators (*J. Biol. Chem.* **157,** *423* (*1945*)) demonstrated that a highly purified L. casei factor (Hutchings, Stokstad, Bohonos, and Slobodkin, *Science* **99,** *371* (*1944*)) was a potent vitamin M.

Similarly, it had become apparent that Bc, a substance necessary for hematopoiesis in the chick, also possessed microbiologic activity (*Nutrition Reviews* **2,** *131* (*1944*)). Concomitantly, it became evident that L. casei factors were potent in the cure and prevention of anemia and leukopenia which occur in rats treated with sulfonamides (see Daft and Sebrell, *Vitamins and Hormones* **III,** *49* (*1945*)). It appeared logical, therefore, to test the clinical activity of various vitamin M substances in anemias, especially in macrocytic anemias such as sprue and

pernicious anemia which are accompanied by hyperplastic marrow, and in leukopenias and agranulocytosis.

In a preliminary paper Spies, Vilter, Koch, and Caldwell (*South. Med. J.* **38,** *707 (1945)*) have reported the hematologic responses following the administration of the synthetic L. casei factor discussed previously to 9 cases of unspecified macrocytic anemia. The synthetic compound was dissolved in saline made alkaline with sodium bicarbonate and was administered intravenously in total doses of 20 to 360 mg. over one to eighteen days. Five hospitalized patients "with macrocytic anemia in relapse" were so treated. Meats and meat products were withheld from the diet. Four of the patients had previously responded to liver extract; the fifth had not been previously treated. Daily erythrocyte, reticulocyte, and leucocyte counts, and hemoglobin determinations were made. Maximum reticulocyte responses of 6.5 to 15 per cent were observed on the sixth to tenth day. "A slight increase in the number of red blood cells and hemoglobin content occurred in each case." One case showed a second reticulocyte response to another prolonged treatment with the L. casei factor. An additional 4 cases responded with a reticulocyte peak and an increase in red cell count following the oral administration of 50 mg. doses of the L. casei factor.

This preliminary report gives no diagnostic criteria, and the cases apparently were not classified as either pernicious anemia (the most common type of macrocytic anemia in most clinics in the United States), sprue, or nutritional macrocytic anemias. No studies of the bone marrow were reported.

Darby and Jones (*Proc. Soc. Exp. Biol. Med.* **60,** *259 (1945)*) have reported the response of 2 cases of sprue to the daily intramuscular administration of 15 mg. of the above described synthetic L. casei factor. The diagnostic criteria fulfilled by both cases constituted the presence of glossitis, diarrhea with increased fat in the stools, marked loss of weight, pigmentation of the skin, macrocytic anemia, moderate leukopenia, impaired absorption as indicated by a flat oral glucose tolerance curve with a normal intravenous curve and a flat vitamin A tolerance curve, the characteristic gastrointestinal pattern on x-ray examination, and a low serum carotene content. Both patients had free acid on gastric analysis; this further differentiated them from cases of pernicious anemia. In the first case the reticulocyte count reached a maximun of 15.3 per cent on the ninth day of therapy and simultaneously there was marked increase of platelets. The erythrocyte count rose from 1.76 million per cmm. to 3.88 million per cmm. by the fifteenth day of therapy. The hemoglobin concentration increased from 7.0 g. to 9.5 g. per 100 ml. within the same period. An upward trend in leucocytes occurred. This hematologic response was accompanied by marked general betterment, regeneration of the lingual papillae, subsidence of the diarrhea, and a gain in weight. The second case had shown a reticulocyte response of 16 per cent on the fourth day of therapy, and a subsequent note (Darby, Jones, and Johnson, *Science* **103,** *108 (1946)*) reports a maximum response of 43 per cent on the sixth day. This later note by the Vanderbilt workers adds the following pertinent information: A third case of sprue has exhibited a similar response; the marrow studies on these cases have revealed that the more primitive red blood cells present before treatment had disappeared and there has been a return of the white cell series in the marrow to normal proportions; the first case previously discussed had gained 26 pounds during the six week period following institution of "folic acid" therapy.

Although sprue is characterized by remissions, the magnitude of these reported responses would seem to be too great and too prompt to be fortuitous. Darby, Jones, and Johnson (*loc. cit.*) suggest that this L. casei factor is identical with or very closely allied to the substance in liver extract which

is effective in the treatment of sprue. Furthermore, they feel that one may regard vitamin M deficiency in the monkey as the experimental analogue of sprue in man. These two reports, if extended and verified, constitute an important step in our understanding of another disease which has often been associated with malnutrition.

Before the synthetic L. casei factor was available, Watson, Sebrell, McKelvey, and Daft (*Am. J. Med. Sci.* **210,** *463 (1945)*) had tested the efficacy of an orally administered concentrate containing 5 mg. of the fermentation residue L. casei factor (Hutchings, Stokstad, Bohonos, and Slobodkin, *loc. cit.*) for a six day period in two groups of cases. Eight patients with so-called refractory anemias failed to be benefited by such therapeutic measures. Seven patients with leukopenia following x-ray therapy, however, showed an increase in the white cell count after the L. casei factor was given. It is of interest that there was no response following the factor to a case of leukopenia persisting after sulfonamide therapy. The investigators draw no conclusions from these results, but the findings should serve to stimulate further study of the possible effectiveness of this group of compounds in the treatment of leukopenia, particularly that following radiation.

Berry, Spies, and Doan (*South. Med. J.* **38,** *590 (1945)*) have reported upon the effect of "a special fraction of liver extract containing among other things, folic acid" and of an undesignated "crystalline L. casei factor" supplied by the Lederle Laboratories, Inc., upon "leucocyte equilibrium in malnourished patients." An initial leucocyte count was made at 8:00 a. m. the day of the experimental study. The material to be tested was injected and the white cell counts repeated at hourly intervals until 3:00 to 5:00 p.m. No mention is made of control of activity of these patients or of the many other factors which are known to influence leucocyte counts (Garrey and Bryan, *Physiol. Rev.* **15,** *597 (1935)*). The maximum post-injection count was tabulated with the initial basal (8:00 a.m.) count. Thirteen of 17 patients so examined were found to have some rise in total leucocyte counts by these standards after the injection of the liver concentrate. The 5 patients treated with the "crystalline folic acid" also had white cell counts higher than the initial one. The peak counts occurred in from two to five hours after the injection of either preparation. When the increase occurred it was of the order of 5 to 100 per cent. This rise was not maintained unless therapy was continued on successive days. No marrow studies were reported. The authors conclude that "these findings suggest that in some cases the use of an intravenous liver concentrate containing folic acid will elevate the total number of circulating leucocytes with a proportionate increase in granulocytes for varying periods." Berry, Spies, and Cline (*South. Med. J.* **38,** *656 (1945)*) report a similar response of cells of the granulocyte series following the intravenous injection of 20 to 60 mg. of a crystalline material isolated from the liver preparations "Reticulogen." As far as one can determine from the protocols and charts there was no difference between the response to the liver fraction, the "crystalline L. casei factor," or the "Reticulogen" fraction. The significance of these reports cannot be evaluated until more data covering a longer period of observation are reported.

VITAMIN Bc AND THE HEMATOLOGIC PICTURE IN THE CHICK

Vitamin Bc is one of the liver factors which must be added to a synthetic diet containing all the other known vitamins in order to maintain the normal growth of the chick and to prevent anemia (*Nutrition Reviews* **2,** *55 (1944)*; **4,** *15 (1946)*). Since

this and related factors are of considerable biologic and medical importance a detailed description of the hematologic findings in the chick subjected to a deficiency of Bc is timely.

Campbell, McCabe, Brown, and Emmett (*Am. J. Physiol.* **144,** *348 (1945)*) have conducted studies on chicks using crystalline Bc and have described in detail the changes which occur in the blood picture during a prolonged Bc deficiency. One-day-old white Leghorn chicks were used. The first of three groups of chicks received a normal diet, which consisted of commercial broiler ration, containing 18 per cent protein. The second group was fed a purified diet containing all the known vitamins except Bc. The third group was given the same purified diet and vitamin supplements but received in addition varying amounts of crystalline vitamin Bc.

The chicks on the normal diet grew well. The blood picture remained fairly constant during the twenty-eight day test. There was some increase in lymphocytes. The erythrocytes in the blood had reached full maturity by the end of two to three weeks. In the first two weeks there was a moderate polychromasia and basophilia demonstrable in the nucleated erythrocytes. The one-day-old chicks showed granulation of the leucocytes but this phenomenon disappeared after a few days.

The chicks on the Bc deficient diet developed a characteristic syndrome. After one to two weeks the animals became lethargic and their wings drooped. After two to three weeks symptoms of incomplete feathering developed, and after four to five weeks the enamel on the shaft of the feather gradually disappeared. The mortality amounted to about 50 per cent. During the first week the blood picture remained essentially similar to that of normal chicks. Thereafter, the erythrocyte count dropped progressively from 2 million per cmm. to less than 900,000 (in one case the count was only 300,000). Immature red cells appeared showing basophilia. In the second week there was distinct macrocytosis, and after three weeks normoblasts, pronormoblasts, and myeloblasts showing mitotic figures appeared in the blood. In the latest stages a pronounced anisocytosis developed.

The leucocyte picture did not show abnormal signs until the second week. The disappearance of the granulation of leucocytes during the first days took place in the Bc deficient chicks in the same manner as in the normal chicks. During the second week a pronounced leukopenia developed. This leukopenia affected all types of leucocytes except the heterophiles, the count of which remained unaltered. The heterophile leucocytes later showed a distinct pyknosis and the lymphocytes became vacuolated.

The thrombocyte picture showed signs of abnormalities during the second week in the form of karyorrhexis, pyknosis, and later cytoplasmic vacuoles. The latter change became so pronounced in the third or fourth week that the thrombocytes almost resembled lymphocytes, and showed great variations in size and shape. Thrombocytopenia did not occur.

Supplementation of the deficient diet with 5 to 10 micrograms of Bc per 100 g. of diet failed to prevent the macrocytic anemia, whereas addition of 20 to 40 micrograms of Bc produced a partial improvement of both the anemia and the leukopenia, although the lymphocytopenia persisted. The blood picture in the chicks receiving 100 micrograms of Bc per 100 g. of diet was essentially normal. Less Bc was necessary for maintaining normal red cell volume and thrombocyte count than to maintain normal red cell and leucocyte counts. Addition of an excess of crystalline Bc (400 micrograms per 100 g. of diet) did not give rise to overstimulation of the production of cell elements. The conclusion was reached that 20 to 200 micrograms of the crystalline Bc per 100 g. of basal diet are sufficient for

normal hematopoiesis in the growing chick for the first four weeks of life.

The similarity of the blood changes here reported to the nutritional cytopenia occurring in monkeys as a result of vitamin M deficiency (Day, *Vitamins and Hormones* **II,** *91 (1945)*) as well as the similar properties of Bc, Bc conjugate, and vitamin M lead one to suggest that the nutritional defect in the two species of animals is either identical or closely akin (*Nutrition Reviews* **4,** *11 (1946)*).

SOME RELATIONS OF VITAMIN Bc CONJUGATE

Confusion has arisen in the discussion of a group of substances variously known as "folic acid," "L. casei factor(s)," "norit eluate factor," "growth factor for S. lactis," etc. Greater confusion has appeared in some papers when attempts have been made to relate these microbiologically active substances to nutritional factors required by the monkey, the chick, and the sulfa treated rat. Because of the importance of these materials and because of the rapidity of development of the whole field, the details of isolation and of chemical studies, and in some cases even of the source of these factors has been withheld. Hence, to term all such concentrates "folic acid" and to expect identical physiologic properties from them is illogical at the present time. The term "folic acid" was employed by Mitchell, Snell, and Williams (*J. Am. Chem. Soc.* **63,** *2284 (1941)*) to signify a material obtained from spinach and assayed microbiologically employing S. lactis R. They defined "folic acid" as a growth factor for S. lactis R (*Ibid.* **66,** *267 (1944)*) and this terminology will be adhered to in the following discussion. For the present review some of the factors known in more or less pure state will be discussed and the nomenclature adhered to which was applied by the investigator who reported the activity of the highly purified factor.

The two microorganisms used in testing the activity of these substances are L. casei and S. lactis (identical with S. faecalis). The active concentrates have been prepared from yeast, liver, a "fermentation residue," spinach, and unstated sources. Stokstad (*J. Biol. Chem.* **149,** *573 (1943)*) prepared two L. casei factors, one from yeast, the other from liver. These were equally active for promoting growth of L. casei, but the liver factor was twice as active for S. lactis as was the yeast factor. The following year Hutchings, Stokstad, Bohonos, and Slobodkin (*Science* **99,** *371 (1944)*) reported the isolation from an unstated source of a third L. casei factor, 85 to 90 per cent as active for L. casei as their previous liver factor, but 6 per cent as active for S. lactis. It has been revealed that this factor is from a "fermentation residue" (Angier *et al.*, *Ibid.* **102,** *227 (1945)*). A substance, perhaps related, was isolated from an undisclosed source by Keresztesy, Rickes, and Stokes (*Ibid.* **97,** *465 (1943)*) and was stated to replace "folic acid" in S. lactis assays but to be inactive for L. casei. This factor was stated not to be "folic acid."

Pfiffner and co-workers (*Science* **97,** *404 (1943)*) isolated from liver an orange colored acid which was active for L. casei and also protected the chick against the anemia of Bc deficiency. This material was termed vitamin Bc and its activity for S. lactis was implied. These same workers (*Ibid.* **102,** *228 (1945)*) have now isolated a second crystalline compound with antianemic activity for the chick. It was obtained from yeast and has been termed vitamin Bc conjugate. This new factor has a molecular weight of about two and eight tenths times that of Bc; it apparently possesses the same chromophoric group as Bc. Less pure concentrates of this Bc conjugate from yeast were shown to yield typical vitamin Bc upon

digestion (Binkley *et al.*, *Ibid.* **100,** *36* (*1944*)) with suitable enzyme preparations. Bc conjugate is much less active (less than 1 per cent) for both L. casei or S. lactis than is Bc. The authors state that the microbiologic activity clearly differentiates vitamin Bc conjugate from the two L. casei factors of Stokstad (*loc. cit.*) and the third L. casei factor of Hutchings, Stokstad, Bohonos, and Slobodkin (*loc. cit.*). Incubation of Bc conjugate with "vitamin Bc conjugase" from hog kidney (Bird *et al.*, *J. Biol. Chem.* **157,** *413* (*1945*)) releases microbiologically active vitamin Bc.

The isolation of this conjugate is a definite confirmation of much experimental data which has compared microbiologic activity of less pure materials before and after enzymatic treatment with the efficacy of the materials for animals. Bc conjugate (from yeast), very active for the prevention of anemia in the chick, must be subjected to enzymatic action before it has as great microbiologic activity as does Bc which has been isolated from liver. The enzyme which is responsible for this activation has been termed "vitamin Bc conjugase" by Bird *et al.* (*loc. cit.*) and appears to be identical in action with that preparation which Mims, Totter, and Day (*J. Biol. Chem.* **155,** *401* (*1944*); *Science* **100,** *223* (*1944*)) employed in estimating "potential folic acid." The basis of this statement is that a purified enzyme preparation used by the Arkansas investigators activates Bc conjugate for S. lactis R (Laskowski, Mims, and Day, *J. Biol. Chem.* **157,** *731* (*1945*); Bird *et al.*, *loc. cit.*). This enzyme is incapable of bringing about the production of S. lactis R factor from xanthopterin (Mims, Totter, and Day, *Ibid.* **155,** *401* (*1944*)), so that one may separate this reaction with xanthopterin from the enzymatic activation of Bc conjugate for S. lactis.

There has accumulated much evidence concerning the importance for animal nutrition of some factor present in yeast and other foods but which is not "folic acid" as is strictly defined above. Thus, Elvehjem and associates prepared liver fractions which possessed antianemic activity for chicks but had little influence on growth of L. casei or S. faecalis (Briggs, Luckey, Elvehjem, and Hart, *J. Biol. Chem.* **148,** *163* (*1943*); **158,** *303* (*1945*)). These active factors have been termed vitamin B_{10} and B_{11}. Hill, Norris, and Heuser (*J. Nutrition* **28,** *175* (*1944*)) report a similar relationship for their chick antianemia substances R and S from yeast. Totter and co-workers (*J. Biol. Chem.* **152,** *147* (*1944*)) demonstrated that vitamin M, the factor required by the monkey to prevent nutritional cytopenia, does not parallel the S. lactis R factor, "folic acid," in its distribution in foodstuffs. They pointed out, however, that such results did "not preclude the possibility that 'folic acid' may be one of two or more substances with vitamin M activity." Yeast, rich in vitamin M activity but somehwat low in "folic acid," became very active for S. lactis after incubating with the liver enzyme discussed above (Totter, Mims, and Day, *Science* **100,** *223* (*1944*)). This led these workers to state that "from a nutritional standpoint the determination of 'potential' (preformed plus extra) 'folic acid' may be of much greater significance than the determination of preformed 'folic acid'." Similarly, Mallory, Mims, Totter, and Day (*J. Biol. Chem.* **156,** *317* (*1944*)) demonstrated that for rats fed a diet containing 1 per cent succinylsulfathiazole there was again a dissociation of the true "folic acid" content of supplements and the growth promoting and leucopoietic activity of the supplement for the rat. However, there was good correspondence between the activity for the rat and the content of potential "folic acid" as determined after enzymatic treatment. Likewise, Wright and associates have shown that a similar potential "folic acid" is present in milk (*Nutrition Reviews* **3,** *273* (*1945*)) and Stewart, Daft, and Sebrell,

(see Daft and Sebrell, *Vitamins and Hormones* **III,** *60 (1945)*) reported the existence of a substance active for the sulfa treated rat but with little or no microbiologic activity until treated with acid or alkali. Pfiffner *et al.* (*Science* **102,** *228 (1945)*) suggest that many of these factors which are microbiologically inactive but possess hematopoietic and growth promoting activity will prove to be identical with the newly isolated vitamin Bc conjugate.

These developments justify the following generalizations: There exist in liver and perhaps in other substances a group of physiologically and chemically related factors which are necessary for the growth of certain microorganisms and for hematopoiesis in the monkey, chick, and rat. These compounds may vary in their relative activity for different microorganisms. In yeast and some other substances there also exist some one or more of the above vitamins in a form which is utilizable by animals but not by microorganisms until acted upon by an enzyme which is widely distributed in tissues or by the action of acid or alkali. Although these factors are closely related, it is confusing and unscientific to refer to these substances indiscriminately as "folic acid."

THE EFFECT OF ACID BEVERAGES ON DENTAL HEALTH OF ANIMALS

It was first suggested some fifty years ago (Miller, "The Microorganisms of the Human Mouth," *S. S. White Dental Mfg. Co., Philadelphia (1890)*) that dental caries (tooth decay) is a process of decalcification of the dental tissues due to acids of bacterial origin. *In vitro* studies have shown that devitalized dentine and enamel are attacked by acids. It has been suggested, however, that the buffering effect of the saliva might prevent or minimize tissue destruction in the mouth by acid foods and beverages. Some data are now at hand concerning the effects of soft drinks and other beverages used by large numbers of people on the molar teeth of experimental animals.

McClure (*J. Nutrition* **26,** *251 (1943)*) studied the effect on rats' molar teeth of the regular consumption, in place of drinking water, of representative "soft drinks," certain fruit juices, a dilute solution of hydrochloric acid, and a dilute solution of lactic acid. The pH of these solutions varied from 1.5 to 3.5. In each of the various experiments, litters of weanling rats were distributed among three or more groups. One group in each experiment was allowed distilled water ad libitum, while for each of the other groups there was substituted one of the acid beverages. At the end of specified periods ranging from forty-two to eighty-five days, the animals were sacrificed and each molar tooth was examined.

All of the acid solutions were very destructive to tooth enamel and to dentine. In all animals the lower molars were more severely affected than the upper molars presumably because of more extensive contact with the drinking fluids. Macroscopically the crowns of the lower molars were seen to be severely damaged and microscopically an unmistakable corrosion was apparent. The weights of the lower molars were reduced in some instances by as much as 35 or 40 per cent. Since a large portion of the weight of a tooth consists of unexposed root, it is obvious that this represents a very grave loss of enamel and dentine. McClure called attention to Miller's conception of the etiology of dental caries, i.e. decalcification of dental tissues by acids of bacterial origin. He concluded that an association of the destructive effects of acid beverages with the initiation of dental caries remains to be determined.

Restarski, Gortner, and McCay (*J. Am. Dental Assn.* **32,** *668 (1945)*) have made

similar studies using puppies as well as rats. In addition these workers also compared, in short term experiments, the destructive effect of a 10 per cent sucrose solution, a phosphoric acid solution (0.055 per cent, pH 2.6), a sucrose-acid solution of these same strengths, and the same sucrose-acid solution containing 1, 6, or 20 parts per million of fluorine. The 10 per cent sucrose solution was not found to be injurious to the dental enamel in these experiments. The acid solution containing no sugar appeared to be much less injurious than the acid solution containing sucrose. This was true even when the sugar-free acid solution was sweetened with saccharin and was made equal to the sugar solution in viscosity (by the addition of dimethoxycellulose, "methocel"). The presence of fluorine in the sucrose-acid solution appeared to protect to some extent about three fourths of the animals against damage to the molars. It was stated that no significant difference in protection was noted for the different levels of fluorine tested. The authors attributed the protective effect of the fluorine to a direct action on the enamel surfaces. They based this conclusion on the observation that 20 p.p.m. of fluorine which was present in the ration used permitted enamel destruction by the sucrose-acid solution, yet 1 p.p.m. of fluorine in this drinking fluid (the same diet being used) exerted a protective action.

The data presented by McClure and by Restarski *et al.* demonstrate that certain acid beverages are injurious to the teeth of experimental animals. However, Restarski and co-workers make the following statement: "The findings on rats and puppies do not, at present, justify any definite conclusions with respect to the effects on teeth of acid beverage ingestion by man." In these experiments the acid beverage was the only fluid available to the animals throughout the period of study, a condition considerably different from the use of acid beverages in man and perhaps of importance in the results obtained.

The suggestions of Restarski *et al.* that the presence of sugar may aggravate the harmful effects of the acid beverages and that the destruction of the enamel in this way may be partially prevented by the inclusion of fluorides in the acid solutions need further experimental investigation. It is not entirely clear that adequate numbers of animals were used to establish these points and the results with fluorine are not consistent. For example, a level of 6 p.p.m. of fluorine appears from the data presented to have been ineffective as compared with levels of 1 or 20 p.p.m. The results are nevertheless provocative.

BREAD ENRICHMENT AND FLOUR MILLING

It is of interest to observe the different policies being urged by scientific authorities in different countries with regard to flour milling and bread production. The British government at the beginning of last year reduced the extraction of the wheat grain for the National Bread, and thereby precipitated a good deal of discussion in parliamentary, medical, and scientific circles on the question of future policy concerning bread. The Nutrition Society held a discussion on the nutritive value of bread as human food (*Brit. Med. J.* **1,** *379 (1945)*). It was opened by the chairman (Professor Peters), who praised the National Bread made from flour of 85 per cent extraction and regretted that the extraction rate had recently been lowered. He emphasized that in the present state of knowledge it is unsafe to assume that fractions discarded during milling could be replaced by the introduction of a few known vitamins.

The protein content of various types of flour was discussed by Chick, who had made

measurements indicating the greater biologic value of the proteins of higher extraction flour. Macrae presented evidence that the digestibility of such flours is less than that of white flours, but this does not outweigh the superior biologic value of their proteins. The vitamin value of different types of flour was discussed by Copping, who concluded that in content of the known B-vitamins the difference between whole meal and 85 per cent extraction is trivial, but that as extraction decreased below 85 per cent, the loss of vitamins becomes serious.

A policy of enrichment was advocated by Kent-Jones, and Bacharach suggested the value of a flexible policy of combined high extraction and enrichment which could be varied to suit immediate needs. A report that the introduction of whole meal bread had caused an increase in rickets in Dublin was mentioned, but it was pointed out that there was also a simultaneous shortage of cod liver oil, and Fridericia remarked that in Denmark whole meal rye bread with added wheat offals is freely consumed and the incidence of rickets is low.

The rival claims of man and animals for the coarser fractions of wheat received much attention: Kay pointed out that the cow is independent of the vitamins of such fractions since they are synthesized by bacteria in the rumen, therefore the cow can dispense with such fractions altogether if adequate quantities of protein cake are available. On the other hand Halman suggested that this is not true of pigs and poultry, for which animals wheat offals are a staple.

Williams and McCollum (*Science* **102,** *180* (*1945*)) have had a discussion on this subject, and a "debate" on the merits of enriched bread versus whole wheat bread with McCollum, Wilder, Morgan, and Mitchell participating has been published (*J. Home Econ.* **37,** *397* (*1945*)).

Facts are needed to settle a scientific problem, not opinions, and if anything is clear in this question it is that we require much more exact information before a final conclusion on the relative value of enriched versus high extraction flours for health in man can be reached, but unfortunately no reports are appearing of experiments on man, aimed at resolving the questions at issue.

SOME EFFECTS OF NUTRITION EDUCATION BY THE PUBLIC HEALTH NURSE

A careful analysis of the effectiveness of the nutrition teaching of a group of public health nurses has been presented by Downes (*Milbank Memorial Fund Quart.* **21,** *158*, *164* (*1943*)) and by Downes and Baranovsky (*Ibid.* **23,** *227* (*1945*)). This study was made among Negro families in Upper Harlem, New York City, who were under nursing supervision because of the presence within the family of active or recently active cases of tuberculosis. The visiting nurses also served in the clinic, so they had an intimate knowledge of the patients and their families. The studies were made intermittently from February, 1940 through April, 1944.

Detailed records of social and economic conditions of each family were collected and, in addition, at monthly intervals during the first year a one week record was obtained of the amount of money spent for food, and of the amount or frequency of consumption of milk, eggs, fruits, green and yellow vegetables, meats, fish, and poultry. On the basis of these records the diets were classified as "excellent," "good," "fair," and "poor" according to a recommended dietary pattern based on the Food and Nutrition Board's Allowances. (To the credit of these workers it should be noted that they have employed these standards as goals for obtaining a good dietary, not as measures of deficiency.)

The tabulations of weekly expenditures

for food were made on the basis of "average adult cost unit" during a period in 1940 and again in 1943. In the 1940 study 66 per cent of the families spent less than $3.00 per cost unit per week in food; in 1943 only 8 per cent were spending so little for food. Some 66 per cent were spending from $3.00 to $4.99 per cost unit in the latter period. The average expenditure per cost unit for food during 1943 was greater than the "estimated minimum cost for food" necessary to meet the standards, but during 1940 this average fell short of the estimated minimum in families containing more than 3.75 "adult cost units."

A further study of 215 of these families was made to determine the quality of the diet. From the food records these dietaries were scored by assigning equal weights to each of the food groups. These ratings revealed that the size of the family did not affect the distribution of the quality of food choices. The frequently noted relationsbip existed between the quality of the diet and the amount of money spent for food. However, ample expenditures did not insure good quality diets as shown by the fact that 44 per cent of the family food habits were rated "fair" or "poor" even when the expenditures for food were satisfactory.

One hundred sixty-six of the families were then studied to determine the effect of the nurse's supervision and teaching. The teaching was defined as (1) frequent instruction, (2) some instruction, (3) instruction as to dietary for the patient only, and (4) no instruction. In the group receiving frequent instruction as to proper diet for the family there occurred an increase of 33.3 per cent in the number rated as "good or excellent." With "some instruction, but not frequent" only 6.8 per cent increase in the "good or excellent" groups was noted. When instruction was limited to the patient a 5.6 per cent increase in these groups was observed. In cases given no instruction a 13.6 per cent increase in the favorable groups was recorded. Thus it is evident that the proper nutrition teaching must be directed at the family, not just the patient in order to affect the food habits of the family unit.

Sixty of these Negro families then served as a sample for an experiment in nutrition teaching. The seven-day records were again obtained simultaneously with nutrition instruction from April to June, 1943. Visiting was then discontinued until October when it was resumed, with teaching, for another three months. In April, 1944, another food record was taken to evaluate the permanent effects of the teaching.

The nurse used the original diet record as a guide for placing greatest teaching emphasis. An experienced nutritionist was available for consultation with the nurse on special problems. Instruction was intensive in all families whose diets were even slightly below the standard. Instruction included nutritive values of specific foods, selection of foods, budgets, shopping, care of foods, preparation, menus, and use of ration points. Encouragement to continue good diets was given where the pattern was satisfactory.

The effects of this program may be evaluated by the data on the percentage distribution of the families according to their scored food habits for April, 1943, and April, 1944, respectively, as follows: standard or above 41.7 and 86.7 per cent; slightly below standard 46.7 and 11.6 per cent; marginal 8.3 and 0 per cent; and unsatisfactory 3.3 and 1.7 per cent. Furthermore, in every food group except butter the mean value for use in 1944 was greater than in 1943. Thus it is demonstrated that nurses' nutrition teaching, done on a selective basis, can be effective under such conditions. Obviously, it is desirable for nurses to incorporate such teaching in their programs.

The changes in the whole grain cereal

food group in this study deserve particular comment. Despite the fact that one fourth of the families received no instruction about the advisability of including dark bread or whole grain cereals in the diet, it was usually recommended that the families adopt the use of dark breads and whole grain cereals. The percentage of families having some dark bread at least once a day increased from 47 to 75 during the experimental year. This demonstrates again that the proper educational approach may result in the acceptance of dark breads when such breads are available.

Since the National Nutrition Conference in 1941 there have been disseminated vast amounts of generalized educational material to point out to the public the need for improvement in the dietary habits of the population. This program has been valuable, but it seems likely that it may not have influenced the nutritionally poorest area of the population because this section, in general, is less alert to such an educational campaign than the more favored groups and also because generalized statements of desirable aims fail to point the way for the individual to overcome the obstacle which is preventing him from attaining that goal. Such obstacles may be poverty, lack of knowledge of food values, ignorance of shopping facilities or sources, inability to plan or budget food supplies, lack of skill in the preparation of food, lack of initiative in varying ways of serving the same foodstuff, and many others. Individualized education is essential to success in any effort to alter food habits in such cases. This education must be founded on an understanding knowledge of the individual's problem. There is no worker better fitted to serve this need than the public health nurse.

DIET AND THE MECHANISMS FOR DEFENSE AGAINST BACTERIAL INFECTIONS

It has been a general impression among clinicians that infection finds a more fertile ground in those who are nutritionally deficient than in healthy persons. In Alabama, Riddle, Spies, and Hudson (*Proc. Soc. Exp. Biol. Med.* **45,** *361* (*1940*)) studied 150 patients with pellagra who had definite clinical lesions. Staphylococcus aureus and Streptococcus hemolyticus were isolated from cheilotic and ocular lesions. These organisms disappeared following riboflavin therapy. In gingivitis and stomatitis large numbers of Vincent's organisms were found in addition to the bacteria above mentioned. These disappeared following nicotinic acid treatment. Experimentally, similar observations have been made. Watson, Wilson, and Topley (*J. Hygiene* **38,** *424* (*1938*)) observed a higher susceptibility to natural contact infection in mice on a diet from which dried skim milk was omitted as compared with animals that were well nourished. Previous studies (*Nutrition Reviews* **3,** *78* (*1945*)) indicated that impairment of the antibody mechanism is one of the sequelae of protein undernutrition. Some studies dealing with the influence of nutritional factors on phagocytosis have also been discussed (*Ibid.* **2,** *232* (*1944*)). These workers interpreted their results as indicating that the general nutritional level of their animals influenced the degree of phagocytic activity of the leucocytes. A number of other studies have been made which are concerned with the relationship of nutrition to susceptibility to infection.

Impressed by the frequency of infections among their patients at the Nutrition Clinic of the Hillman Hospital in Birmingham, Berry, Davis, and Spies (*J. Lab. Clin. Med.* **30,** *684* (*1945*)) sought to explore the subject further by placing rats on a diet similar to that consumed by their patients. In such animals the total number of leucocytes, the

various types of leucocytes, the phagocytic activity of the granular leucocytes, and the ability of the animals to produce antibodies were measured.

One hundred Sprague-Dawley weanling rats were divided into ten groups with 10 rats in each group. Five groups were males and five females. Two groups, one of males and one of females, received a basal diet consisting of 35.6 per cent corn meal, 28.1 per cent unenriched white flour, 17.6 per cent pork fat, and 18.7 per cent cane sugar. Two more groups received this basal diet plus 18 per cent vitamin free casein. Two groups received a mineral mixture in addition to the basal diet plus casein; two groups were given the basal diet plus casein and also the B-vitamins, thiamine, riboflavin, pyridoxine, inositol, calcium pantothenate, nicotinamide, and choline. Finally, two groups received the basal diet together with casein, the B-vitamins, and the minerals. Rats receiving the basal diet suffered loss of weight, loss of appetite, and alopecia. Only when the diet was supplemented with high grade protein, vitamins, and minerals was normal development attained.

Phagocytic activity of the granular leucocytes was measured by the technic of Cottingham and Mills to whose work reference was made above. This consists in counting the number of bacteria found in a certain number of neutrophilic granulocytes after a measured amount of a standardized suspension of Micrococcus candidus has been mixed with blood. The ability to form antibodies (agglutinins) was first tested with Bact. tularense as the antigen but when this proved unsatisfactory in control as well as in the deficient animals, a mixed typhoid-paratyphoid vaccine was used. In all of the studies care was taken to control variations in the results which might be caused by differences in handling the animals, the time of taking samples of blood, the density of bacterial suspensions, and various other factors.

The animals were permitted to remain on the diets for two months before any tests were begun. Of the various experimental groups growth was poorest in the animals receiving the basal diet, best in those receiving the basal diet supplemented with casein, minerals, and vitamins, and intermediate between these extremes in the groups receiving the basal diet plus casein, basal diet plus casein and minerals, and basal diet plus casein and vitamins, in the order given.

The leucocyte counts in both the male and the female rats receiving the basal diet were found to be only a third of those in the well fed, normal groups. The average counts were 5963 and 4980 in males and females, respectively, and ranged from 2100 to 6650 as compared with averages of 15,250 and 14,216 with a range of 12,250 to 18,500 in the animals receiving the best diet. The leucocyte counts in the other groups were intermediate between these extremes and on the average were all lower than those of the adequately fed animals. The investigators refer to a shifting in the differential leucocyte picture toward a relative increase in polymorphonuclear neutrophils with a corresponding relative decrease in lymphocytes and an absolute decrease in all cellular types. The data, however, are less convincing in this respect.

With regard to agglutinin production, it was found that the rats receiving the basal diet had titers on the average only 23.3 per cent as high as the well fed males and 45.1 per cent as high as the well fed females. Thus, the average agglutination titer for typhoid-paratyphoid vaccine in the animals receiving the basal diet was 1:2390 for the males and 1:7368 for the females with ranges from 1:320 to 1:10,240. In the best fed group the averages were 1:10,240 in the males and 1:16,336 in the females with a range of 1:10,240 to 1:20,480. Less striking differences were observed in the intermediate groups.

The granular leucocytes of the rats on the basal diet engulfed only 60.6 to 64.2 per cent of the number of bacteria which the

control animals were able to engulf. The average figure in the former groups was 8.94 bacteria phagocytized per cell in the males and 15.99 in the females as compared with 14.74 and 23.35 in males and females respectively of the best fed group. Statistical evaluation indicated that these differences between the animals receiving the basal diet and those given the best diet were significant.

These studies tend to support the hypothesis that resistance to bacterial infections may be depressed by inadequate nutrition. Attention is called by the investigators to the fact that these observations differ from those made in regard to virus infections. Thus, in studies previously reviewed (*Nutrition Reviews* **3,** *69* (*1945*) thiamine deficient mice were found to be more resistant to the virus of poliomyelitis than well nourished animals. The differences in findings are not necessarily contradictory, however, for the defense mechanisms against bacterial diseases may be controlled by factors which are different from those concerned with virus infections.

FATTY ACID METABOLISM

The science of nutrition falls roughly into three stages, the third (and longest?) of which is just beginning. During the first stage, investigations were directed chiefly to ascertaining the proximate composition of foodstuffs, the consequences of disproportionate consumption of these components of the diet —carbohydrates, fats, proteins, salts—and, in a general way, the metabolism of these major dietary components. In the second stage, the importance of trace substances was established, and the findings were so dramatic that the earlier metabolic questions received somewhat abated attention. This second stage may nearly have run its course. Although new vitamins will undoubtedly be discovered, it seems likely that for many animals at least the majority of the vitamins and essential trace minerals have been ascertained. In the third stage, which has indeed already begun, one may anticipate the establishment of a more intimate picture of the metabolism of carbohydrates, fats, and proteins in terms of enzymatic transformations and degradations, in which many of the vitamins and trace minerals will be expected to appear as prosthetic groups of certain of the enzymes concerned. It seems likely, therefore, that the nutrition and metabolism of the proximate components of the diet will be emphasized once more. Hence it is with considerable interest that one reads the review of Stadie on the present status of knowledge concerning the metabolism of fatty acids (*Physiol. Rev.* **25,** *395* (*1945*)).

During the past few years there has been a considerable revolution in certain of the concepts of fat metabolism which had been dominant for several decades. Stadie presents the evidence that makes these concepts no longer tenable. For years the theory of successive beta-oxidation had been almost universally accepted. According to this theory fatty acids in the animal organism are degraded 2 carbon atoms at a time until a 4 carbon particle is left, which in the normal organism may then be completely oxidized, or, in the diabetic animal, be converted into ketone bodies. It now appears to be well established that the fatty acid molecule can be converted almost completely into ketone bodies. Thus a mol of palmitic acid with 16 carbon atoms may yield as many as 4 mols of acetoacetic acid, instead of one as called for by the original theory. Furthermore, the ketone bodies, rather than arising as the abnormal consequence of a deranged metabolism, are almost certainly produced in large amounts by the normal liver. Since, however, the muscles, heart, kidney, etc. can normally utilize these large amounts as fast as they are produced, ketosis

does not ensue. It is only when fat metabolism is greatly accelerated as in diabetes and starvation that ketone production exceeds the rate of utilization with consequent rise in the ketone body concentrations in the blood and urine. Thus the liver, by partially oxidizing the fats to acetoacetic and betahydroxybutyric acids, prepares important metabolites for the use of the rest of the body.

Another long held concept of fat metabolism is that a definite amount of carbohydrate (about two thirds of a gram) must be oxidized per gram of fat in order to prevent ketosis. An obligatory coupling between fat and carbohydrate oxidation was postulated. Such a view is no longer compatible with the evidence. As indicated above, the presence or absence of ketosis is determined by the rate of fat oxidation and the ability of the periphery to consume ketone bodies rather than by any "ketogenic:antiketogenic" ratio. It is nevertheless true that oxidation of adequate amounts of carbohydrate prevents ketosis, but this effect is apparently a consequence of a lowered fat metabolism. How the oxidation of carbohydrate can inhibit fat metabolism remains to be elucidated, but it is suggested that products of carbohydrate metabolism may compete for enzymes which would otherwise degrade fat.

The pathways by which fatty acids are converted into acetoacetic or betahydroxybutyric acids have not been established. The fatty acids might be degraded 2 carbons at a time with rapid resynthesis into 4 carbon compounds (the beta-oxidation-condensation hypothesis of MacKay (*J. Clin. Endocrinology* **3,** *101 (1943)*) or else degraded 4 carbon atoms at a time directly to ketone bodies. Recent evidence, obtained by the use of isotopic carbon as a tracer, strongly supports MacKay's hypothesis (Weinhouse, Medes, and Floyd, *J. Biol. Chem.* **155,** *143 (1944)*).

The failure to find metabolic intermediates in fat oxidation has been somewhat baffling. Expected intermediates such as acetic acid and fatty acids with less than 12 carbon atoms have never been demonstrated in tissues in spite of serious search for them. Other metabolic pathways have been proposed which would not require these particular intermediates, but again, since the requisite intermediates have not been found, the situation is not much improved. Apparently the intermediate oxidative steps are so rapid that there is no significant accumulation of intermediate products.

A major portion of Stadie's review is devoted to a discussion of enzyme systems concerned with fat metabolism. This interesting portion cannot be detailed here, but on reading this material one is impressed by the fact that although the enzyme chemistry of fats appears to be rather rudimentary in comparison with that of carbohydrates or even proteins, there has been so much recent progress that this disparity may soon disappear. It is in this intermediate zone of investigation that the older and newer stages of nutritional science join, and will, let it be hoped, come to early fruition.

THE DIAGNOSIS OF BERIBERI HEART DISEASE

In view of the isolated case reports wet beriberi seems to be uncommon in this country. This may result from true rarity, or from failure either to recognize the syndrome or to report it in medical journals. Indeed, contemporary texts on diseases of the heart pay little attention to it. Three reasons for this state of affairs are suggested by Blankenhorn (*Ann. Int. Med.* **23,** *398 (1945)*). First is lack of a pathognomonic lesion or group of lesions at autopsy; second, the pathogenesis is not comprehensible to the physiologist or cardiologist in dynamic terms; and finally the descriptive terminol-

ogy of the Oriental disease, perhaps a compendium of many different disorders, still dominates the somewhat cloudy picture. On the other hand precise knowledge of many formerly obscure types of heart disease and a rapidly increasing body of facts about deficiency diseases offer a background against which wet beriberi should become more intelligible.

Blankenhorn has suggested eight criteria for the diagnosis of the cardiac form of beriberi: (1) enlarged heart with normal sino-auricular rhythm, (2) dependent edema, (3) elevated venous pressure, (4) peripheral neuritis or pellagra, (5) nonspecific changes in the electrocardiogram, (6) lack of other recognized cause of heart failure, (7) grossly deficient diet for at least three months, and (8) clinical improvement with reduction of heart size after specific treatment, or autopsy findings consistent with beriberi according to the criteria of the American Heart Association (1939). On the basis of these diagnostic points 10 patients seen during the last four years in the Cincinnati General Hospital were judged to have beriberi. It is of great interest to see how poorly they fit into the traditional picture of the cardiac or wet variety of beriberi. The circulation was rapid in only 5 of the 8 patients studied by the decholin method. Only half of the patients had clinical evidence of an enlarged right heart. "Pistol shot" heart sounds were heard only twice. The evidence for rapid blood flow therefore was meager. In Blankenhorn's experience rapid flow was not a late sign. In some alcoholics it occurred without congestive failure or other signs of beriberi. Gallop rhythm occurred in 4 patients, shock or syncope in 5. Only one showed typical response to large doses of thiamine (20 to 50 mg. daily for at least two weeks).

It is surprising that no mention is made of plasma protein or its component fractions since one of the classic causes of edema following inadequate food is directly attributable to hypoproteinemia. Furthermore, although true beriberi may have existed in persons recently liberated from Japanese and German prisons and concentration camps it was often so mixed with starvation edema that the diagnosis was rarely made.

Another finding in the Cincinnati cases was the failure to respond to adequate digitalis therapy. This has been observed by others (Weiss and Wilkins, *Ann. Int. Med.* **11,** *104 (1937)*; Weiss, *J. Am. Med. Assn.* **115,** *832 (1940)*) although its significance is not clear. Careful study of this phenomenon might help to reveal the mechanism by which beriberi is produced.

Blankenhorn's data and conclusions should serve to reopen the entire question of wet beriberi. A critique of the problem is hardly justified by the present information. Contemporary methods in cardiology and nutrition, particularly the biochemical developments now reaching perfection must be applied to the problem. Only when a collaborative study of the numerous facets of the disease has been made will it be possible to define beriberi, to establish unimpeachable criteria for its diagnosis, and measure the effectiveness of therapy. The present report serves to reopen the question and to focus attention on a neglected problem.

THE ADRENAL CORTEX AND PROTEIN METABOLISM

It has been postulated that the adrenal cortex has an important function in nitrogen metabolism particularly in regard to the formation of carbohydrate from protein. If this is correct, it is not unreasonable to expect adrenal hypertrophy to follow the ingestion of a high protein diet. Several groups of investigators have carried out experiments designed to test this supposition but have obtained results which are some-

what conflicting. It appears that some high protein diets do result in adrenal hypertrophy but that others do not.

Long, Katzin, and Fry (*Endocrinology* **26,** *309* (*1940*)) found that in adrenalectomized rats and mice subjected to fasting the glycogen levels declined more rapidly than in normal animals. When cortical extract (or in some experiments crystalline corticosterone or dehydrocorticosterone) was injected into normal or adrenalectomized animals which were either fed or fasted, abnormally high levels of liver glycogen resulted. In mice, with intact adrenals, which had been fed during the injection period, the levels were particularly high, as much as 12 per cent of the weight of the liver in individual animals being made up of glycogen. These results are in general agreement with those of several earlier investigators and indicate that the adrenal cortex participates in the hormonal control of carbohydrate metabolism.

Long and co-workers also presented evidence which seems to indicate that increased protein catabolism is involved in the physiologic process leading to this excessive glycogen deposition. It had been noted previously that fasted adrenalectomized rats excreted about 25 per cent less nitrogen than did fasted normal rats. Long *et al.* reported that rats treated with cortical extract or cortical hormones showed increased nitrogen excretion and that the increase paralleled the level of glycogen formation. The glucose:nitrogen ratios for normal and adrenalectomized rats were 3.3 and 4.1, respectively, indicating that 53 and 66 per cent of the extra protein catabolized was converted into glucose. This suggests that the control over carbohydrate metabolism exercised by the adrenal cortex involves specifically the formation of carbohydrate from protein. Long *et al.* postulated that one of the properties of the cortical hormone is a stimulation of protein catabolism, and that the increased carbohydrate levels and the increased nitrogen and potassium excretion following its injection into animals is an expression of this effect.

A great deal of attention has been paid in recent years to adrenal hypertrophy as a "reaction to stress" or as an "alarm reaction." Tepperman, Engel, and Long (*Endocrinology* **32,** *373* (*1943*)), after a survey of the pertinent literature, pointed out that in a great many conditions of so-called "stress" and in others which are not usually classified as stress but are followed by adrenal hypertrophy, there is an increase in the proportion of protein in the metabolic mixture. They suggested that a substrate derived from the catabolism of either tissue or dietary protein may serve as the stimulus for hypertrophy of the adrenal cortex in a variety of circumstances. As pointed out by the authors, it is necessary to differentiate sharply between cases where increased protein catabolism might be thought to call forth a greater output of hormone and cases where increased amounts of hormone, injected or resulting from the administration of other hormones, might cause an increase in protein catabolism.

The hypothesis that increased protein catabolism will cause adrenal hypertrophy has been tested experimentally by three groups of investigators. Tepperman, Engel, and Long (*Endocrinology* **32,** *403* (*1943*)), using rats, compared the effects on adrenal size of a diet consisting of raw ground beef (55 per cent protein) and two diets containing 60 and 78 per cent, respectively, of casein, with a control diet consisting of Purina fox chow (24.4 per cent protein). The three high protein diets caused a 40 to 50 per cent increase in adrenal size above that of the controls in two to seven weeks. This increase appeared to be primarily cortical in nature. Ingle, Ginther, and Nezamis (*Ibid.* **32,** *410* (*1943*)), also using rats, compared the effects of high protein, high carbohydrate, and high fat diets. The protein content of these diets

ranged from approximately 15 to 70 per cent, in the form of yeast and casein. The observed differences in adrenal weights were small and the authors concluded that they were probably without significance. Ingle *et al.* also compared the effect of stress, which consisted of continuous stimulation of the gastrocnemius muscle for a period of one hundred twenty hours. The weights of the adrenals of these animals were much greater than those of the animals fed a high protein diet and were approximately double those of control animals. Benua and Howard (*Ibid.* **36,** *170 (1945)*) employed mice as experimental animals and tested a variety of diets containing from approximately 15 to 80 per cent protein in the form of casein and yeast. They found no significant differences in adrenal size between the various groups. Ingle (*Ibid.* **37,** *7 (1945)*) reinvestigated the question, using some diets similar to those previously employed by Ingle *et al.* and others similar to those of Tepperman and co-workers. The results of these experiments at least partially confirmed the findings of both groups of investigators. No differences in adrenal weights resulted from the diets of Ingle and co-workers but certain of the high protein diets of Tepperman *et al.* did cause adrenal hypertrophy. From these data it appears that some but not all high protein diets will have this effect. An explanation for this is not at present apparent.

The point of particular interest touched upon by these experiments is the postulate that the hormones of the adrenal cortex play a particular role in the formation of carbohydrate from protein. The failure of adrenal hypertrophy to follow the ingestion of certain high protein diets is not fatal to this theory since, as recognized by the various workers in the field, the exact relationship between hypertrophy and cortical activity is uncertain. It is more difficult to reconcile this postulate with the observation of Ingle and co-workers that adrenalectomized rats may live and show excellent growth on a high protein diet without supplementary treatment with cortical hormones. As stated by Ingle this objection is not incontrovertible and the hypothesis deserves further testing.

METHIONINE IN THE TREATMENT OF TOXIC HEPATITIS

An increase in sensitivity of the liver to toxins when the body has been partially depleted by its protein stores has been demonstrated by Miller and Whipple (*Am. J. Med. Sci.* **199,** *204 (1940); J. Exp. Med.* **76,** *421 (1942)*) and the general problem has been discussed in this journal (*Nutrition Reviews* **2,** *292, 340 (1944)*). These workers have suggested that the well known value of carbohydrate in a similar protective role may indeed depend upon its protein sparing capacity. Following the announcement of the protective action of protein there was an intensive search for the fraction or fractions chiefly responsible for it. Methionine, and to some degree cystine, proved to be the most active amino acids. Much additional evidence has verified the original observations for animals whose livers were subject to injury with such different hepatotoxins as chloroform, mapharsen, dichloroethane, and carbon tetrachloride.

During the recent period of extensive manufacture of trinitrotoluene a certain amount of exposure to this agent has been inevitable. The availability of methionine for clinical trial in toxic hepatitis was a fortunate circumstance according to the report of Eddy (*Am. J. Med. Sci.* **210,** *374 (1945)*) who describes the clinical course of 30 cases of acute hepatitis which followed exposure to trinitrotoluene. In spite of the fact that 10 of the cases had severe damage to the liver and about a third of

the reported cases have been fatal there were no deaths in the series of patients treated with methionine. Even though no controls were used, the clinical and laboratory studies on the individual patients before and after methionine therapy was instituted gave convincing testimony that improvement was more than a chance circumstance. It should be emphasized, however, that the use of a diet rich in protein and carbohydrate, poor in fat, and supplemented with multiple vitamins prevents any critical evaluation of methionine as an isolated therapeutic agent in these cases.

Particular effort was made to follow the course of each patient by daily determinations of urinary urobilinogen and bilirubin which appeared to be a more labile guide to the patients' progress than the icterus index. In fact between values of 10 and 20, the icterus index, because of its slow changes, was of no practical value. The cephalin flocculation test was used routinely and appeared to be of more diagnostic value than in prognosis or in estimating the value of therapy. From the case reports it appears that the dosage employed may be significant, for in two instances a dose of 1 g. three times daily appeared ineffective but a dose of 2 g. four times a day was followed by satisfactory improvement of what had appeared to be a critical relapse.

Mention is made of similar improvement following administration of methionine for hepatitis following carbon tetrachloride, and for early cirrhosis or acute relapse in advanced cirrhosis.

This report may be taken as testimony of the value of methionine in toxic hepatitis in man. It does not constitute definitive proof because the evidence is circumstantial, though consistent. Except in so far as each patient was his own control, and the group of cases is compared with others reported in the medical literature, no controls were employed. If further work confirms the suggestions put forward by this investigation it has clearcut implications in industrial hygiene and preventive medicine.

STARVATION: 1945

Modern global warfare leaves in its wake certain small gains in knowledge such as come from systematic starvation of races or classes which has been practiced for inspiring terror or eliminating minorities. While such a policy is not new in the long history of war, its end results have not been studied heretofore in the light of modern knowledge of nutrition.

Stare (*Nutrition Reviews* **3,** *225 (1945)*) has described the circumstances under which a number of survey teams made both preliminary and detailed investigations of the nutritional status of the population of Western Holland immediately upon the liberation of this region by the Allied Armies. Although the comprehensive report on observations and experiences during German occupation and following relief will not be submitted by the various investigators until early in 1946, Burger, Sandstead, and Drummond (*Lancet* **II,** *282 (1945)*) have prepared a preliminary statement on the clinical features and treatment of mass starvation in Western Holland.

When the teams arrived in May and June the average daily caloric intake per person was about 1000 calories, half of which was forage, such as tulip bulbs, sugar beets, and marigolds which invalids were unable to obtain. By the third week after liberation the calories were up to 2000. Five per cent of the population were in a serious state of starvation, which was especially bad in urban areas.

Physical and mental exhaustion, dizziness, and a tendency to collapse after standing were universal, while headache, aching

legs, numbness and tingling of extremities, a sense of chilliness, and nocturia were common. Emaciation, pallor, petechial bleeding, and muscular atrophy were evident upon examination. Hunger edema was not as frequent as cachexia. Cyanosis, loss of elasticity, and gangrene indicated the abnormal state of the skin. Neither scurvy nor full blown pellagra was seen. A low systolic blood pressure and a slow heart rate were characteristic. The body temperature often was low with a tendency to loss of the thermoregulation of normal persons when the environmental temperature changed. A nonbacillary diarrhea occurred in many cases. Many patients were anemic, the usual hemoglobin being about 11 g. per 100 ml. with a tendency to hyperchromia. Leukopenia was noted. Osteoporosis and bone dystrophies were seen. In those who did not survive, death occurred sometimes precipitously, sometimes unexpectedly when recovery seemed assured, and sometimes there was a gradual lapsing into coma.

Treatment was based on absolute bed rest for the very ill, while the less gravely affected persons were kept ambulatory. A comparison of several types of protein hydrolysates and various food mixtures indicated that foods produced the best results if they could be tolerated. Hydrolysates sometimes led to vomiting, did not readily relieve hunger, and required the consumption of much fluid. In many instances feeding of hydrolysates plus glucose by stomach tube improved the patients so that they were able to eat normally, but equally good results were obtained with unhydrolyzed proteins. Extreme degrees of emaciation or deep collapse indicated an irreversible state of the tissues and responded to no therapy.

Diarrhea was treated with kaolin, opium, or sulfonamides. Plasma and whole blood transfusions were not particularly helpful in collapse. Digitalis and mercurial diuretics helped in heart failure but did not affect hunger edema. Thiamine did not influence the edema. Persistent hypotension was ameliorated by use of adrenal cortical extract. The psychoses usually responded to forced feeding. Vitamins, liver extract, and iron did not relieve the anemia which gradually improved after sufficient feeding. Ascorbic acid did not influence the petechial hemorrhages which slowly disappeared under general treatment. Niacin seemed to reduce dermal pigmentation and niacinamide relieved the painful red tongue which was a frequent sequel to diarrhea. Focal osteoporosis improved readily with injection of vitamin D_3 while the generalized type responded more slowly.

A critical analysis of this work by the numerous physicians collaborating in it cannot be made until the detailed reports are available. Those responsible for the program are to be praised for the systematic observations made on the clinical aspects of starvation and on the various therapeutic and dietetic measures to relieve it. From the data on the terminal phases of starvation it is of singular interest that vitamin deficiency disease syndromes were rare, while most of the trouble seemed to come from the lack of calories and protein. When the whole story is told more of the clinical vagaries of nutritional disturbances will undoubtedly become less obscure.

NITROGEN ABSORPTION, NITROGEN BALANCE, AND THE BIOLOGIC VALUE OF PROTEINS

A full discussion of the questions posed in experiments to determine the biologic value of proteins and protein mixtures was reviewed previously in this journal (*Nutrition Reviews* **2**, *169* (*1944*)). Further discussion of some aspects of the problem is now pre-

sented in a paper by Allison and Anderson (*J. Nutrition* **29,** *413 (1945)*). They refer to the usual definition of the biologic value of a protein as the fraction of absorbed food nitrogen which is retained in the body of the animal. They also discuss the objection to using this value which arises from the fact that in order to compute it one must assume that a constant excretion of "endogenous" nitrogen occurs.

Allison and Anderson however point out that basically this concept of biologic value is a function of the relationship between nitrogen balance and nitrogen intake, and further that this relationship can be used to evaluate dietary proteins without any further assumptions. Melnick and Cowgill (*J. Nutrition* **13,** *401 (1937)*), in determining the minimal protein intake for nitrogen equilibrium in dogs, measured this relationship over a limited range of intake. They observed that in the region of nitrogen equilibrium there is a linear relation between the nitrogen balance and the percentage of protein calories in the diet. In the present study an attempt was made with normal adult dogs to establish the relationship more clearly over a wider range of values.

In their experiments a protein free diet similar to that of Melnick and Cowgill was used. The constituents were sucrose, dextrin, glucose, lard, salt, and agar, with addition of an adequate supplement of vitamins. The dogs ordinarily received enough of the diet to supply 80 calories per kilogram daily; when protein was added, equicaloric amounts of carbohydrate were omitted, and when natural foodstuffs containing carbohydrate and fat were used, an equal amount of fat and carbohydrate was omitted from the basal diet. The dependence of nitrogen balance on absorbed nitrogen (both expressed as grams of N per square meter per day) was studied on 6 dogs and over a wide range of nitrogen balances.

When the results for each dog were plotted graphically, it appeared that from the most extreme negative balances observed up to small positive balances, the relation to absorbed nitrogen was linear; but at moderate to large positive balances the linear relationship no longer existed, and the nitrogen balance increased less rapidly with respect to increased absorption. Through the region of linear relationship, the graph could be described by the equation

$$\text{NB (nitrogen balance)} = \text{K} \times \text{AN}$$

$$\text{(absorbed nitrogen)} - \text{NE}_0$$

where NE_0 is the excretion of nitrogen on the protein free diet. In this equation the empirical constant K describes the slope of the line relating nitrogen balance to absorbed nitrogen, but the authors show that if the assumption is made that the "endogenous" excretion of nitrogen in the feces and urine is constant, then the constant K describes that fraction of the absorbed nitrogen which is retained in the body of the animal—that is, the biologic value of the protein.

Other experiments demonstrated that the graph lines obtained when diets with a given protein were fed in different quantities were separate but parallel, thus showing that the biologic value of a protein is independent of the caloric intake. The level of nitrogen absorption needed to maintain equilibrium differed from one dog to another, although the graph lines for different dogs were parallel; however, it is suggested that if the caloric intake is sufficiently high, this difference is decreased and may almost disappear.

In these experiments Allison and Anderson used several different proteins for which they report values of K as follows: casein 0.82; cooked chicken entrails 0.77; cooked flounder entrails 0.77; cooked flounder heads 0.52; and a soybean protein 0.39.

NOTES

Letters to the Editor

Dear Sir:

In a recent issue of your journal (*Nutrition Reviews* **3,** *175 (1945)*) there appeared an article on "Serum Albumin Regeneration by Protein Hydrolysates," dealing with the paper by Cox and Mueller (*J. Clin. Invest.* **23,** *875 (1944)*), in which it was concluded that the hydrolysates were of equal value. However, both the original article and the review state that "It is well known that ingested proteins vary considerably in their ability to cause regeneration of plasma albumin." This, of course, is true when comparisons are made between proteins of high nutritional value and those known to be deficient in certain essential amino acids. However, Cox and Mueller employed in their study hydrolysates of beef serum protein, lactalbumin, and casein, all being of good nutritional value. Unfortunately Cox and Mueller cited, among others, the paper by Melnick, Cowgill, and Burack (*J. Exp. Med.* **64,** *897 (1936)*) in support of the above quoted statement. Actually, the paper by Melnick and associates presents experimental evidence for the opposite conclusion, viz., that "The three complete proteins, serum protein, lactalbumin, and casein are approximately equal with respect to their value in meeting the requirements of nitrogen equilibrium. Likewise they are of comparable value in promoting regeneration of serum protein."

Responsibility rests primarily upon the studies from Whipple's laboratory for the reports on widely divergent potency ratios. This work has been criticized because of the many uncontrolled variables which were allowed to operate (cf. Melnick, Cowgill, and Burack, *J. Exp. Med.* **64,** *877 (1936)*). Madden and Whipple (*Physiol. Rev.* **20,** *194 (1940)*) dismiss the papers coming from the Yale laboratory on the alleged ground that the reserve store of plasma protein-building materials had not been completely removed during the depletion periods, without referring to the paper of Melnick and associates (*loc. cit.*) which deals specifically with this question and presents experimental support for the reliability of the assay technic employed.

These points are raised because we feel that although the results reported by Cox and Mueller are correct, no difference exists in relative utilization of hydrolysates compared with intact proteins. These points have been brought to the attention of Dr. Cox who regrets the improper reference to our work and states, "Our paper would have been materially strengthened if we had indicated that your prior study had shown that the feeding of serum protein, lactalbumin and casein to experimental animals resulted in an equivalent regeneration of total serum protein."

Sincerely yours,
Daniel Melnick,
Food Research Laboratories, Inc.,
Long Island City, New York.

Symposium on Nutrition

A symposium (*Va. Med. Mo.* **72,** *238 (1945)*) presenting a survey of the role of nutrition in various branches of medical practice has been reported. In discussing nutrition in relation to internal medicine, Porter placed particular emphasis on the well recognized role of vitamins in deficiency disease, the protection which special dietary constituents may afford against bacterial and chemical poisons, and the intimate connection between parasitic diseases and nutrition. Sutton presented a general review of the effects and manifestations of nutritional deficiencies in obstetrics and pediatrics, and

Riggin discussed the role of nutrition in preventive medicine and public health.

In considering relations of nutrition to surgery, Evans has emphasized the need of measuring total circulating plasma volume rather than concentration alone in determining protein needs either preoperatively or postoperatively. Protein deficiency and unnecessary operative risks may actually exist with normal blood plasma concentrations.

Youmans closed the symposium with a discussion of many of the problems which have been worked upon intensively during the war—the curious phase of negative nitrogen balance following surgical operations and injuries, the diagnosis of various stages of clinical and early malnutrition, the use of laboratory tests of vitamin stores and excretion rates. A detailed consideration was given for the discrepancies between the data obtained by careful dietary history, clinical examination, and laboratory findings, especially biochemical estimates of vitamins.

The Ascorbic Acid Content of Milk as Consumed

The loss of vitamins in processing, shipping, storing, cooking, and serving of foods constitutes a variable which may range from great importance to insignificance. Light, air, heat, and moisture singly or in combinations cause deterioration in the nutritional value of various foods. A study by Mawson and Kon (*Lancet* **II**, *14* (*1945*)) on the ascorbic acid content of milk as consumed is devoted to analysis of the stages and degrees of loss from milk pail to consumer's table.

Samples were collected from the breakfast or lunch milk and analyzed within one hour. Reduced as well as total ascorbic acid was determined. On the average the consumer received only half of the fairly constant 2 to 2.5 mg. of ascorbic acid contained in 100 ml. of fresh, raw milk.

As expected with lower temperatures and reduced light intensity, January samples contained somewhat more ascorbic acid than August samples. Raw stored milk usually contained about two thirds of the original ascorbic acid whereas heated milk contained only one-third. By obtaining samples at various stages between production and consumption it was found that relatively small losses occurred after milk was delivered, and these were negligible unless it was kept overnight.

The authors attribute much of the loss from heated milk to increased handling in processing and delay in delivery which is made safe by virtue of pasteurization but which entails more exposure. Wartime measures necessitated certain practices which undoubtedly will be corrected with increased availability of labor. This anticipated change plus the use of a more rapid method of pasteurization which has little effect on ascorbic acid should reduce the losses considerably.

Without such studies as this, data concerning vitamin content of fresh unprocessed foods lose much of their meaning. Indeed they may lead to a false sense of security—a belief in the presence of substances which have disappeared. Tables of nutrient content should if possible indicate expected processing losses.

Recent Nutrition Books

Vitamins and Hormones. Vol. III. Edited by Kenneth V. Thimann and Robert S. Harris. Academic Press, Inc., New York. 1945. Pp. 420.

Laboratory Manual of Field Methods for Biochemical Assessment of Metabolic and Nutritional Condition. Harvard Fatigue Laboratory, Boston, Mass. 1945. Pp. 132.

Food Famine and Nutritional Diseases in Travancore (1943–44). Servindia Kerala Relief Centre, R. S. Puram Post Coimbatore, S. India. 1945. 265 pp. (33 illustrations).

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